Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action

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Overview of the Effects of β-Adrenergic Receptor Agonists on Animal Growth Including Mechanisms of Action[1,2,3,4]

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ABSTRACT: The β-adrenergic receptors (β-AR) are present on the surface of almost every type of mammalian cell. These receptors are stimulated physiologically by the neurotransmitter, norepinephrine and the adrenal medullary hormone, epinephrine. There are three subtypes of β-AR, namely, β_1-AR, β_2-AR, and β_3-AR; the pharmacological and physiological responses of an individual cell result from the particular mixture of the three β-AR subtypes present on that cell. Species-specific structure (amino acid sequence) also causes modification of the function of a given β-AR subtype. Knowledge of the β-AR subtypes present in various cell types, coupled with knowledge of receptor structure (sequence), will allow an understanding of the complexity of physiological function regulated by β-AR. Oral administration of some β-AR agonists increases muscle and decreases fat accretion in cattle, pigs, poultry, and sheep. The large number of physiological functions controlled by β-AR suggests that the mechanism(s) for the observed changes in carcass composition may be extremely complex. Any proposed mechanism must begin with the possibility of direct effects of the agonist on skeletal muscle and adipocyte β-AR. However, many other mechanisms, such as modification of blood flow, release of hormones, or central nervous system control of feed intake may contribute to the overall effects observed with a given β-AR agonist in a given species. Furthermore, the pharmacodynamic properties of a particular agonist are complex and expected to vary among species as well as within the same species at different ages or when fed different diets.

Key Words: β-Adrenergic Agonists, β-Adrenergic Receptors, Adipose Tissue, Skeletal Muscle, Growth

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Introduction

A physiological response is produced when a β-adrenergic agonist binds to a β-adrenergic receptor (**β-AR**). The physiological β-AR agonists are norepinephrine and epinephrine. Oral administration of some synthetic β-AR agonists causes modification of growth with increased accretion of skeletal muscle and decreased accretion of fat. There are three β-AR subtypes, the β_1-AR, the β_2-AR, and the β_3-AR. The β-AR are present on most mammalian cells, but the distribution of subtypes and proportion of each varies between tissues in a given species. The β-AR subtype distribution also varies within a given tissue between species. Finally, the amino acid sequence varies for a given β-AR subtype across species. As a consequence of the variation in receptor subtype structure and distribution across tissues and species, the multitude of physiological effects controlled by β-AR, and the use of several different agonists, the mechanisms operative to produce the pharmacological effects observed with oral administration of a β-AR agonist are complex and difficult to discern. This review describes

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[2]This review is not intended to be comprehensive. The literature cited encompasses other reviews, articles not included in previous reviews, and articles necessary to document a particular point. The literature cited dates through June 1, 1996.

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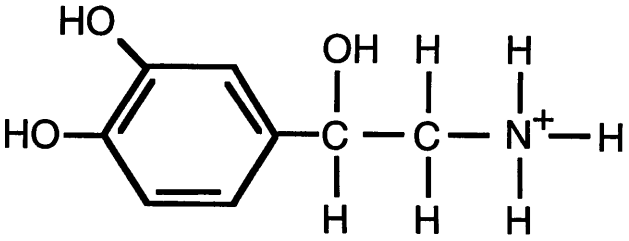
aspects of the modulation of animal growth by β-AR agonists and some of the proposed mechanisms leading to such effects.

β-*Adrenergic Receptor Agonists*

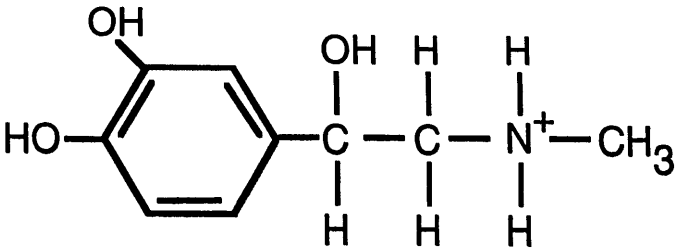
The β-adrenergic receptor agonists are organic molecules that bind to β-adrenergic receptors such that the agonist-receptor complex activates the G_s protein. The α-subunit of the G_s protein then activates adenylyl cyclase, the enzyme that produces cyclic adenosine monophosphate (**cAMP**), one of the major intracellular signaling molecules. The mechanism by which cAMP produces effects is to bind to the regulatory subunit of protein kinase A to release the catalytic subunit that then phosphorylates a number of intracellular proteins. Some of these proteins are enzymes that are activated when phosphorylated (e.g., hormone sensitive lipase, the rate-limiting enzyme for adipocyte triacylglycerol degradation). The cAMP response element binding protein (**CREB**) is phosphorylated by protein kinase A; the CREB binds to a cAMP response element in the regulatory part of a gene and stimulates the transcription of that gene. Phosphorylation increases the transcriptional activity of the CREB, providing the mechanism for β-AR agonist-mediated transcription of a number of genes in the mammalian cell. Other enzymes become inactivated when phosphorylated (e.g., acetyl-CoA carboxylase, the rate-limiting enzyme for long-chain fatty acid biosynthesis (Mersmann, 1989a; Strosberg, 1992; Liggett and Raymond, 1993).

The physiological β-AR agonists are norepinephrine and epinephrine (Figure 1). Norepinephrine, the catecholamine sympathetic nervous system neurotransmitter molecule, is biosynthesized from tyrosine; it also circulates in the serum at relatively high concentrations. The catecholamine epinephrine is synthesized in and secreted from the adrenal medulla; it circulates at lower concentrations than norepinephrine in most mammalian species, but during stress it usually responds to a greater extent than norepinephrine. Epinephrine is biosynthesized from norepinephrine and is the methylation product of norepinephrine.

Extensive interest in β-AR in the biomedical community led to the synthesis of thousands of organic molecules that bind to β-AR; some of these ligands are agonists, whereas some are antagonists (bind to the receptor but do not activate the G_s protein and thus block the receptor function). Much of the interest in β-AR agonists and antagonists has focused on the production of compounds that have relative specificity to stimulate the β-AR of bronchial-tracheal musculature, causing relaxation and dilation of the airways (relieve asthma), or that have relative specificity to change cardiovascular function by moderating heart rate, contractility, or blood pressure.



Norepinephrine



Epinephrine

Figure 1. Structure of the physiological β-AR agonists. Norepinephrine is the neurotransmitter substance released at central nervous system and sympathetic nervous system nerve endings; it also circulates in the plasma at relatively high concentrations. Epinephrine is the hormone produced by the adrenal medulla and released to the plasma.

It is necessary to dispose of receptor agonists in an efficient manner so the receptor does not remain activated. Norepinephrine and epinephrine are inactivated by catechol-o-methyl transferase, an enzyme that methylates the catechol-ring hydroxyl groups, and by monoamine oxidase, an enzyme that deaminates the ligand. After release from sympathetic nerve endings, norepinephrine may be reabsorbed by specific reuptake mechanisms (at synaptic clefts and myoneural junctions) to decrease the concentration at the effector site (Mersmann, 1989a; Hoffman and Lefkowitz, 1990; Landsberg and Young, 1992).

Use of β-*Adrenergic Receptor Agonists in Growing Animals*

Cunningham (1965) presented data that indicated the possibility of changing mammalian growth by administration of agents (e.g., caffeine, theophylline, nicotine, and epinephrine) that directly or indirectly might function by changing the intracellular concentration of cAMP. In the early 1980s, workers at American Cyanamid Co. published data on the modulation of growth in animals fed clenbuterol, a β-AR agonist (Ricks et al., 1984). In growing cattle,

chickens, pigs, and sheep, oral clenbuterol increased muscle mass and decreased fat mass. In some cases, there was also an increase in weight gain and in the gain-to-feed intake ratio. In the following years, several other β-AR agonists (e.g., cimaterol, ractopamine, L 664,969, salbutamol) were fed to various species; effects similar to those of clenbuterol were produced. To date, none of these compounds has governmental approval to modify growth of animals raised for meat production in the United States or in any other country.

A simplified summary of the effects of oral β-AR agonists in several species is presented in Table 1. There are extensive summaries of the effects of these compounds on avian and mammalian growth (Hanrahan, 1987; Beermann, 1989, 1994; Anderson et al., 1991; Bergen and Merkel, 1991; Moloney et al., 1991; Wellenreiter, 1991; McKeith et al., 1994; NRC, 1994; Steele et al., 1994); the Moloney treatise is particularly comprehensive.

The effects of β-AR agonists are not as pronounced in chickens as they are in sheep; the effects in pigs are intermediate, and in cattle, the effects tend to be somewhat as in sheep. One possible reason for major species differences in response to β-AR agonists is that some species have been intensely selected for growth rate and have less potential to increase growth because they are closer to the biological maximal growth rate (e.g., broiler chickens). Other species (e.g., sheep) have not been so intensely selected for growth rate and thus have more potential for increased growth rates. Another reason for species differences is that a particular β-AR agonist may not be as effective in one species as in another. This type of disparate response may result because a given agonist may not activate the target tissue β-AR as well in one species as in another; possible mechanisms include agonist affinity for the receptor(s), coupling of the agonist-receptor complex to the signal transduction system, and factors that influence delivery of the compound to the receptor sites. In addition, the β-AR in target tissues may be rapidly inactivated, or a particular species may have a limited number of β-AR on target tissues, reducing the response to the agonist.

Although attempts have been made to compare quantitative responses (the efficacy and potency) of several agonists in a single species, this type of comparison is valid only when the agonists are simultaneously tested in a dose × response manner in the same experiment. There are far too many tangible and intangible factors in animal growth experiments and in drug trials to draw comparative conclusions from different individual experiments.

β-*Adrenergic Receptors*

Almost every mammalian cell type has β-AR embedded in the plasma membrane. These receptors have > 400 amino acids in a continuous chain. Models for the β-AR (Figure 2) indicate seven relatively hydrophobic transmembrane domains that anchor the receptor in the plasma membrane. There are four extracellular portions on the outside of the membrane (three loops connect adjacent transmembrane domains) and four intracellular portions on the inside of the membrane (three loops connect adjacent transmembrane domains). The ligand binding site is in the center of the seven transmembrane domains and involves amino acids from several of the domains. The sites for interaction with the G_s protein have been localized to portions of intracellular loops 2, 3, and 4. To avoid indefinite activation of the β-AR, either the agonist may be removed by reuptake mechanisms or degraded, as already mentioned, or the receptor may be inactivated by several mechanisms. After binding of the agonist, the β-AR may be phosphorylated by a specific kinase, the β-AR kinase at sites localized in intracellular loop 4. Phosphorylation inactivates the receptor. Protein kinase A can also phosphorylate the receptor. The β-AR may be removed from the plasma membrane during conditions of chronic stimulation to decrease the response by a reduction in available β-AR (Ostrowski et al., 1992; Schwinn et al., 1992; Strosberg, 1992; Kobilka and Hoffman, 1995).

β-*Adrenergic Receptor Subtypes*

Functional Classification. The systematic investigation of physiological functions stimulated or inhibited by norepinephrine, epinephrine, and a few other compounds led to the concept of α-adrenergic receptors (α-AR) and β-AR in the late 1940s. Norepinephrine and epinephrine stimulate α-AR and β-AR, but epinephrine is more potent (effective at lower concentrations) than norepinephrine for α-AR. Twenty years later, the β-AR were subclassified into β_1-AR and β_2-AR. Norepinephrine is more potent for β_1-AR than for β_2-AR. The classification schemes allow better comprehension of the complexities of adrenergic function. They also led to the use of prototypical tissues (i.e., a tissue that predominantly has a single adrenergic receptor subtype as evidenced by its responses in the classification studies). Examples of prototypical tissues are rat heart for β_1-AR responses and guinea pig tracheal musculature for β_2-AR responses. The prototypical tissues were subsequently used to classify large numbers of individual agonists or antagonists regarding their specificity for the receptor subtypes. A few compounds are relatively specific for a receptor subtype; these compounds were used to classify β-AR subtypes in tissues and species other than the prototypical ones. Evidence for a third β-AR subtype, the β_3-AR, began to appear in the mid-1970s.

Ligand Binding Classification. In the mid-1970s, the technique of measuring ligand binding to the β-AR was developed (Williams and Lefkowitz, 1978). This approach has been used to supplement the measurement of physiological function for determination of

Table 1. Effects of oral administration of β-adrenergic receptor agonists to mammals and birds[a]

Animal	Weight gain	Feed consumption	Gain/feed	Muscle	Fat
Cattle	+10	–5	+15	+10	–30
Chickens	+2		+2	+2	–7
Pigs	+4	–5	–5	+4	–8
Sheep	+15	+2	+15	+25	–25

[a]The values are indicated as the percentage change. These approximations are adapted from Moloney et al. (1991); the original article tabulates data from numerous trials for each species, for several β-adrenergic receptor agonists, with multiple doses of agonist. As expected, the results are highly variable, including some negative responses.

receptor pharmacology and receptor classification. Ligands are designated as specific for a particular receptor subtype on the basis of high affinity for prototypical tissue receptors; receptors in other tissues are then classified by the binding of subtype specific ligands. Ligand binding more directly assesses the receptors than does measurement of physiological function; however, it does not predict physiological function, because it does not measure coupling to the cAMP-protein kinase A pathway, and it does not distinguish between agonists and antagonists. Thus, a physiological approach is also needed to understand the function of a ligand; in many cases, activation of adenylyl cyclase is measured as an expression of physiological function (Hoffman and Lefkowitz, 1990; Landsberg and Young, 1992; Mersmann, 1995).

Ligand-binding experiments that use a wide range of ligand concentrations with sufficient intermediate concentrations can generate evidence for multiple binding sites. These methods indicate prototypical tissues have a large number of the particular β-AR subtype suggested by physiological approaches (e.g., rat heart has > 90% β_1-AR, whereas guinea pig tracheal musculature has > 85% β_2-AR). However, most tissues, including prototypical tissues, have more than one β-AR subtype (Minneman et al., 1979). In a given species, the proportion of β_1-AR and β_2-AR is different in individual cell types or tissues (e.g., human tissues in Table 2). In the example (Table 2), the proportion of the two β-AR subtypes in a given tissue is the same whether determined using a β_1-AR specific antagonist (CGP 20,712A) or a β_2-AR specific antagonist (ICI 118,551) in competitive ligand-binding experiments. The affinity of the β_1-AR antagonist for the high-affinity site (K_H) is similar for all tissues; the affinity of this ligand for the low-affinity site (K_L) also is similar for all tissues. Because this antagonist has been designated as specific for β_1-AR based on previous experiments with prototypical tissues, the high-affinity site is designated as the β_1-AR. When the β_2-AR antagonist is used, it detects two binding sites and the K_H and K_L for all tissues are similar; in this case the high-affinity site (K_H) is designated the β_2-AR. These data are unusually clear (Table 2). In other species or when other ligands are used, the data from such experiments may not be so definitive because the agonists or antagonists may not have such ideal binding properties with the β_1-AR or β_2-AR present in that species or tissue (Mersmann, 1995).

β_3-Adrenergic Receptor. Beginning in the mid-1970s and extending into the 1980s, evidence continually accumulated from pharmacological studies of rat adipocyte function and ligand binding indicating the β-AR in this tissue was different from the β_1-AR and the β_2-AR (Arch and Kaumann, 1993). This atypical β-AR became the focus of considerable effort because it was also present in rat brown adipose tissue; brown adipose tissue was being intensely investigated as the source of thermogenesis and thus as a major factor in obesity. Although interest in brown adipose tissue as providing a mechanism for obesity in humans has waned, this research led to the discovery of the β_3-AR,

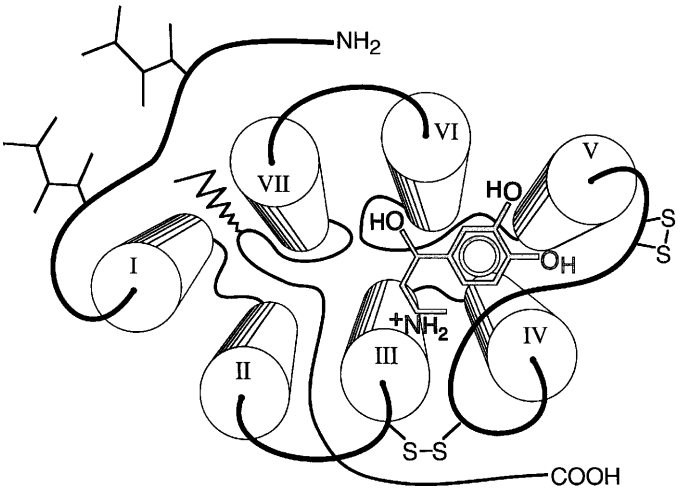


Figure 2. Projected structure of a β-adrenergic receptor. Seven transmembrane domains (cylinders), ligand binding (norepinephrine), extracellular portions (thick lines at top of cylinders) and intracellular portions (thin lines at bottom of cylinders) are indicated. From Ostrowski et al. (1992). Reproduced, with permission, from the Annual Review of Pharmacology and Toxicology, Volume 32, 1992, by Annual Reviews Inc.

Table 2. Relative concentration of β_1- and β_2-adrenergic receptors (AR) in human tissues[a]

Item	Right atrium	Fat	Kidney	Lung	Liver
Competition by CGP 20712A (a β_1-AR antagonist)					
β^1-AR, %	50	35	32	27	18
β_2-AR, %	50	65	68	73	82
K_H, n*M*	1.3	2.7	4.4	2.0	3.8
K_L, n*M*	1,017	700	1,042	1,797	1,546
Competition by ICI 118,551 (a β_2-AR antagonist)					
β_1-AR, %	50	40	36	27	21
β_2-AR, %	50	60	64	73	79
K_H, n*M*	7.1	0.7	2.3	1.9	1.9
K_L, n*M*	1,032	1,222	1,122	1,013	2,142

[a]Adapted from Sano et al. (1993). The percentage of β_1-AR, β_2-AR, and the affinity of each subtype were obtained by competitive ligand binding. The K_d for each receptor site is indicated as a high affinity site (K_H) or a low affinity site (K_L).

the predominant β-AR in brown and white adipose tissue in rats. It is also present in some regions of the gut, and perhaps in skeletal and cardiac muscle. The β_3-AR is pharmacologically distinct from the other two subtypes, and its structure in the fourth intracellular loop provides few sites for inactivation by phosphorylation (Strosberg, 1992; Arch and Kaumann, 1993; Emorine et al., 1994; Giacobino, 1995; Langin et al., 1995).

There are specific agonists for the β_3-AR (Arch and Kaumann, 1993; Howe, 1993), and recently the first antagonist has been identified (Nisoli et al., 1996). An interesting characteristic of the β_3-AR is that several antagonists for β_1-AR and β_2-AR are partial or in some cases full agonists for the β_3-AR. Lafontan and Berlan (1993) classified a number of mammalian species regarding their white adipocyte lipolytic response to isoproterenol, a universal β-AR agonist, BRL 37,344, a designated β_3-AR agonist, and CGP 12,177, a β_1-AR and β_2-AR antagonist but β_3-AR agonist. Some species respond to all three compounds equally, whereas some have a graded response with isoproterenol > BRL > CGP, and other species essentially only respond to isoproterenol. The data suggest that either the β_3-AR in the adipocytes of various species has pharmacological properties distinct from the prototypical rat receptor or that the β_3-AR may not be the predominant receptor subtype in adipocytes in some species (Lafontan, 1994; Langin et al., 1995).

Classification from Molecular Biology. Beginning in the mid-1980s, molecular biology techniques verified the existence of individual β-AR subtypes with distinctly different-sized RNA transcripts, distinct protein sizes, and unique amino acid sequences. Although β-AR subtypes have been sequenced from only a few species, the results indicate the three β-AR subtypes have approximately 50% homology in amino acid sequence within a single species, whereas an individual β-AR subtype has 75% or more homology across species (Strosberg, 1992; Hall et al., 1993; Pietri-Rouxel and Strosberg, 1995). As more β-AR subtypes are sequenced in diverse mammalian and non-mammalian species, the homology relationships will undoubtedly be modified.

The membrane-bound β-AR are difficult to isolate and rather unstable when removed from the membrane. As a result, and because there is so much similarity in structure between β-AR subtypes, the study of protein structure has been of limited use to elucidate structure and function of β-AR. Most of what is known about structural features involved in the function of these receptors has been obtained from molecular biology approaches to detect transcripts, to compare sequences, and to discover functional groups by sequence analysis and by selective site-directed mutation. A summary of the properties of the three β-AR subtypes is presented in Table 3.

Modulation of Subtypes. There are numerous examples of differential desensitization of the three β-AR subtypes by various mechanisms; the β_3-AR are generally less responsive to desensitization than the other two subtypes, and there is some evidence for the β_2-AR being somewhat more readily desensitized than the β_1-AR (Lafontan, 1994; Langin et al., 1995; Marullo et al., 1995).

The β-AR subtype population may change with the stage of differentiation of a cell or with the hormonal milieu provided to the cell. A dramatic example of both these phenomena is observed in the clonal 3T3-F442A cell line maintained in culture. These fibroblast-like cells multiply in culture and after confluence differentiate into adipocytes when presented with appropriate medium components. There are approximately 90% β_1-AR, some β_2-AR, and no β_3-AR on the undifferentiated cells, whereas after differentiation into adipocytes there are approximately 90% β_3-AR, some β_1-AR, and very few β_2-AR. If the differentiation medium contains dexamethasone, the cells have > 80% β_2-AR (Feve et al., 1991; Strosberg, 1992).

Table 3. Properties of β-adrenergic receptors (AR)

Item	β_1-AR	β_2-AR	β_3-AR
Prototypical tissue	Rat heart	Hamster trachea	Rat adipocyte
Selective agonist	—	Fenoterol	CGP 12,177
Selective antagonist	CGP 20, 712A	ICI 118,551	SR 59,230A
Glycosylated molecular weight	65,000	65,000	65,000
No. of amino acids (human)	477	413	408
mRNA, approx. kb	2.8	2.0	2.2
Introns	No	No	Yes
Phosphorylation sites	Yes	Yes	Few or none

Skeletal Muscle and Adipocyte β-*Adrenergic Receptor Subtypes.* Knowledge of the proportion of β-AR subtypes present on a particular cell type in a particular species may suggest the response of that cell to a particular β-AR agonist. In regard to the major species raised for meat production in the developed countries, there is only limited knowledge of the β-AR subtypes on skeletal muscles or on adipocytes, the primary tissues responding to oral β-AR agonists (Mersmann, 1995). In cattle, competitive ligand-binding studies suggest there are predominantly β_2-AR on skeletal muscle and adipocytes (Sillence and Matthews, 1994), whereas other investigators suggest there are approximately 75% β_2-AR and 25% β_1-AR on adipocytes (Van Liefde et al., 1994). Saturation analysis indicates only β_1-AR + β_2-AR sites, but it does not distinguish between them (Sillence and Matthews, 1994; Houseknecht et al., 1995). At this time, there is no evidence for β_3-AR on bovine adipocytes using binding analyses or the measurement of lipolysis (Van Liefde et al., 1994). In sheep, competitive ligand-binding studies indicate predominantly β_2-AR on adipocytes (Bowen et al., 1992). In the bovine and ovine studies, the data are clear, but limited, because each study uses only one β_1-AR ligand (CGP 20,712A) and one β_2-AR ligand (ICI 118,551). Thus, the data do not exclude the possibility that the results represent the affinity of these two compounds for the species-specific receptor present in bovine or ovine adipocytes rather than the detection of β_1-AR and β_2-AR.

There are transcripts for β_1-AR, β_2-AR, and β_3-AR on bovine adipocytes (Casteilla et al., 1994); the β_3-AR transcripts may reside in the few brown adipocytes remaining scattered in the white adipose tissue of the adult after the neonatal period. The β_3-AR is the predominant transcript in the brown adipose tissue of the bovine fetus; this receptor has been cloned and sequenced (Pietri-Rouxel et al., 1995).

In pigs, saturation ligand binding indicates only one site (apparently β_1-AR + β_2-AR) for skeletal muscle; the ligand used probably would not detect β_3-AR (Spurlock et al., 1993a). For porcine adipose tissue, measurement of lipolysis has never clearly indicated the β-AR subtypes, even though a large number of agonists and antagonists has been tested. Saturation ligand binding and competitive ligand binding also with numerous ligands have not clearly indicated the receptor subtypes (Mersmann, 1989c, 1995; Mills and Mersmann, 1995). In some studies using competitive ligand binding with a large number of ligand concentrations, multiple binding sites are evident (Coutinho et al., 1992; Mills and Mersmann, 1995). However, when several β_1-AR specific and several β_2-AR specific ligands are used, there are no clear conclusions regarding which subtype is being detected or the proportion of β_1-AR and β_2-AR present (Mersmann et al., 1993). Ligand-binding studies aimed specifically at detection of the β_3-AR in porcine adipocyte membranes provide little evidence for functional receptors of this subtype; if present, the porcine adipocyte β_3-AR has binding properties quite different from the prototypical rat β_3-AR (Mersmann, 1996).

The mRNA transcripts for β_1-AR, β_2-AR, and β_3-AR are all present in porcine adipocytes (McNeel and Mersmann, 1995); the data are qualitative only and thus do not suggest the proportion of each receptor subtype expressed. The lack of distinctive pharmacology for the β-AR subtypes in porcine adipocytes (both from measurement of lipolysis and ligand binding) and the lack of antibodies for the receptor subtypes preclude any conclusions regarding expression of functional β-AR subtypes on these cells.

Mechanisms

Any predicated mechanism for the effects of β-AR agonists must begin with activation of the β-AR and proceed through the G_s proteins to activation of adenylyl cyclase to produce cAMP. Only after mechanisms mediated by β-AR are thoroughly exhausted can speculation about other mechanisms be entertained. The almost universal distribution of β-AR on all mammalian cell types provides the milieu for complex mechanisms of action depending on the population of β-AR subtypes expressed on various cells and the distribution of the agonist to various tissues. The mechanism of a β-AR agonist in vivo may be extremely entangled with some or even most of the ultimate effects resulting from secondary events caused by hormonal or physiological responses of numerous tissues to the β-AR agonist administered.

Regardless, the effect of the β-AR agonist almost certainly results from activation of β-AR and not by some "magical" function. Because of the similarity in the structure of α-AR and β-AR, it is possible there may be some activation (or inactivation) of α-AR by a particular β-AR agonist. This possibility cannot be totally excluded when exploring mechanisms in vivo but could be indicated as less probable, if testing of the β-AR agonist for α-AR activity in vitro is negative. It is also possible for a β-AR agonist in one species to act as an antagonist for a β-AR in another species and tissue (i.e., it would bind to the receptor, but would not activate adenylyl cyclase; Mills and Mersmann, 1995). Finally, it may be difficult to measure a small perturbation in the rate of a metabolic function over the minutes or hours of an experiment, but a very small change in vivo over the weeks or months of drug administration could easily bring about major changes in the size of a muscle, or a fat depot, or the overall metabolic activity of an organ.

Skeletal Muscle. One of the most obvious effects of orally administrated β-AR agonists in cattle, pigs, and sheep is an increase in muscle mass. Because the postnatal growth of skeletal muscle is primarily a result of hypertrophy, it is expected that an increase in muscle protein synthesis, a decrease in muscle protein degradation, or a combination of both will produce the β-AR agonist-stimulated increase in muscle mass. (Some of the data on skeletal muscle have been reviewed: Yang and McElligott, 1989; Moloney et al., 1991; Kim and Sainz, 1992; Mersmann, 1995.) In individual experiments with various β-AR agonists fed to several species, an increase in protein synthesis and a decrease in protein degradation have been demonstrated. There are also reports that do not indicate changes in rates of protein synthesis or degradation, perhaps because these are difficult measurements and the changes are small (selected references are Bergen et al., 1989; Claeys et al., 1989; Adeola et al., 1992a). Protein degradation is seldom directly measured; instead, protease activities often are measured in muscle from treated animals. Several protease activities often are reduced, or concentration of protease inhibitors is increased, by β-AR agonist treatment (e.g., Wang and Beermann, 1988; Kretchmar et al., 1990; Koohmaraie et al., 1991; Bardsley et al., 1992; Sainz et al., 1993). In pigs infused with epinephrine, data suggest a positive relationship between plasma epinephrine and skeletal muscle calpastatin (the inhibitor of the calcium-activated proteases, the calpains) concentrations (Sensky et al., 1996).

Experiments with muscle in vitro are as troublesome as those in vivo; there are negative and positive results. Muscle preparations in vitro are almost universally not in a physiological state, but a catabolic condition (Mersmann, 1995). Finally, the status of other endocrine systems may influence the observed effects (e.g., some of the variability in experiments attempting to demonstrate skeletal muscle responses to β-AR agonists may depend on the glucocorticoid status of the animal; Liu et al., 1994b).

Treatment of mammals with β-AR agonists causes an increase in the amount of RNA transcript for several skeletal muscle proteins. Thus, the mRNA for myosin light chain (Smith et al., 1989), α-actin (Helferich et al., 1990; Koohmaraie et al., 1991; Grant et al., 1993), and the calpain protease inhibitor calpastatin (Higgins et al., 1988; Bardsley et al., 1992; Killefer and Koohmaraie, 1994) are increased after β-AR agonist treatment.

The density of β-AR present in bovine longissimus and semitendinosus muscles is not correlated with any growth trait (Hoey et al., 1995). Although the response of a cell type or tissue to a β-AR agonist should depend on the receptor number, receptors move on and off the membrane, are inactivated by phosphorylation, and the physiological response usually represents activation of only a few of the receptors (i.e., there are many spare β-AR).

Adipose Tissue. The other obvious effect of oral β-AR agonists is a decrease in carcass fat mass. These agonists clearly stimulate adipocyte triacylglycerol degradation and inhibit fatty acid and triacylglycerol synthesis in vitro in cells or tissue explants from several species. However, with specific agonists and adipocytes from a particular species, negative experiments are sometimes reported. Even agonists that cause a decrease in fat when fed and that bind to the β-AR may have minimal effects on lipid metabolism measured in vitro in adipocytes from that same species (Spurlock et al., 1993b, 1994; Mills and Mersmann, 1995). In some, but not all, cases, after chronic administration of an agonist, adipose tissue from that animal will have increased lipolytic or decreased lipogenic rates measured in vitro. (Many of these studies have been summarized in Mersmann, 1989c, 1990, 1995; Mills and Mersmann, 1995). There has been little investigation of lipid anabolic and catabolic processes in vivo. The elevation of plasma nonesterified fatty acid concentration after administration of a β-AR agonist suggests activation of the adipocyte lipolytic system. Several β-AR agonists acutely elevate plasma nonesterified fatty acid concentration in pigs (Mersmann, 1987; Hu et al., 1988; Adeola et al., 1992b) and in cattle (Blum and Flueckiger, 1988; Eisemann et al., 1988) The response is blunted with chronic administration of the β-AR agonist in cattle (Eisemann et al., 1988) and in sheep (Beermann et al., 1987).

The adipose tissue effects may not be as persistent as the skeletal muscle effects. In multiple studies with pigs fed ractopamine, carcass fat is reduced (e.g., Watkins et al., 1990; Crome et al., 1996). However, one recent investigation indicated that ractopamine, when fed to pigs, had little or no effect on carcass fat

deposition or on adipocyte lipogenic rates measured in vitro (Liu et al., 1994a). Another investigator reported ractopamine caused a decrease in carcass fat content but little or no decrease in the daily rate of lipid deposition (Dunshea et al., 1993a,b). Other recent investigations indicate a decreased rate of lipid deposition in pigs fed ractopamine (Mitchell et al., 1991) or salbutamol (Oksbjerg et al., 1996). It is not possible to decipher the differences between these more recent studies on lipid deposition rates in pigs fed β-AR agonists. In bovine males fed L644,969, protein deposition rates are increased and fat deposition rates are decreased (Chwalibog et al., 1996).

As indicated later in this review, subtleties of experimental design, including genetic background of the animals, might be expected to produce divergent quantitative results and occasionally negative results. The multiple mechanisms to lower the responsiveness of the cells to chronic agonist administration should cause inactivation or removal from the cell surface of β_1-AR and β_2-AR, but perhaps to a lesser extent β_3-AR. Skeletal muscle β-AR are decreased in rats fed cimaterol (Kim et al., 1992) and tend to decrease in pigs fed ractopamine (Sainz et al., 1993; Spurlock et al., 1994). However, the adipose tissue β-AR decrease to a greater extent than skeletal muscle β-AR in pigs (Spurlock et al., 1994). This differential desensitization of porcine adipose tissue and skeletal muscle β-AR may be a mechanism to yield the lesser response of adipose tissue than skeletal muscle observed in some studies.

Other Mechanisms. A number of less direct mechanisms of β-AR agonist activity in vivo readily could contribute to the mechanism of action of an orally administered β-AR agonist. Because of the multiple cell types with β-AR on their surface, it is probable there are multiple effects of a particular β-AR agonist in the recipient animal. Most of these possibilities and the evidence for them were previously discussed and will only be summarized (Buttery and Dawson, 1987; Mersmann, 1989a, 1995; Zimmerli and Blum, 1990; Moloney et al., 1991).

Certainly, β-AR agonists can increase blood flow to certain regions of the body. An increase in blood flow to the skeletal muscle may enhance the process of hypertrophy by delivery of increased amounts of substrates and energy sources for protein synthesis. Likewise, increased blood flow to adipose tissue might be envisioned to carry nonesterified fatty acids away from the tissue to enhance the lipid degradation process. These mechanisms could readily augment the more direct effects of β-AR agonists on the muscle cell and the adipocyte. There is increased blood flow in mammals administered β-AR agonists; to the hindlimb of cattle with acute and chronic agonist administration (Eisemann et al., 1988), to the hindlimb of sheep with chronic agonist administration (Beermann et al., 1987), and to skeletal muscle and adipose tissue with acute administration in pigs (Mersmann, 1989a). Blood flow (not measured) is probably increased to many organs because of the increase in heart rate observed with a number of β-AR agonists (e.g., Mersmann, 1987).

Another possible mechanism is modulation of the circulating concentration of numerous endocrine substances; for example, an acute increase in plasma insulin, but no change after chronic β-AR agonist administration in cattle (Blum and Flueckiger, 1988; Zimmerli and Blum, 1990) or even a decrease in insulin in sheep after chronic β-AR agonist administration (Beermann et al., 1987). Chronic β-AR agonist administration increases plasma thyroid hormones in sheep (Beermann et al., 1987), but not in cattle (Zimmerli and Blum, 1990). Acutely administered exogenous β-AR agonist elevates endogenous plasma catecholamines in pigs; the endogenous catecholamines could then mediate effects in various tissues (Mersmann, 1989b). A similar experiment in cattle (Blum and Flueckiger, 1988) did not modify plasma epinephrine or norepinephrine concentrations.

There is little or no evidence that β-AR agonists increase muscle mass and decrease fat mass by way of somatotropin. First, the β-AR and somatotropin receptors have no structural relationship to each other. Second, there is no evidence the intracellular signaling systems for the two receptors are related. Third, somatotropin produces considerable hypertrophy of many organs, whereas the β-AR agonist-produced hypertrophic effects are restricted to skeletal and probably cardiac muscle and salivary glands (Reeds and Mersmann, 1991). Somatotropin also has profound effects on feed intake, causing a major reduction, whereas the β-AR agonist effects on feed intake generally are small or do not exist. Oral or i.v. administration of β-AR agonists does not seem to increase the secretion of plasma somatotropin, and the agonists actually suppress plasma somatotropin concentrations in sheep (Beermann et al., 1987; Zimmerli and Blum, 1990; Thomas et al., 1994).

The rates of β-AR controlled metabolic pathways in various tissues may be altered by exogenous β-AR agonists; this could result in modified plasma concentrations of metabolites such as glucose or lactate. There is little evidence that chronically administered β-AR agonists increase basal metabolic rate (e.g., Rikhardsson et al., 1991; Yen et al., 1991). The real possibility exists that a systemic β-AR agonist can traverse the blood-brain barrier to produce central nervous system effects as demonstrated for clenbuterol in rats (Ordway et al., 1987). This mechanism could account for the reduced feed intake with some β-AR agonists in some species and in some trials.

Each of the individual mechanisms, or more likely some combination of them (as well as others not mentioned), could be operative in a given species administered a given β-AR agonist, at a particular

age, with a specific genetic background, under a designated husbandry condition, and fed a particular diet. If the agonist is delivered to target tissues at an appropriate concentration and those target tissues have β-AR responsive to the agonist, then a β-AR mediated function of that tissue will contribute to the overall mechanism in that species, with that specific agonist, under the prescribed conditions.

Restrictions on Interpretation and Extrapolation of Experimental Observations

Experiments In Vitro. Many observations are obtained with tissue, cell, or subcellular preparations in vitro. Although little progress would be made in biological research without experimentation in vitro, such results may be misleading when extrapolated to the organism in vivo. First, most tissue or cell preparations are in a catabolic state when studied in vitro. This is true of adipocyte and muscle cells in almost all cases and is clearly demonstrated by the greater rates of catabolic processes such as lipolysis in adipocytes compared to anabolic processes such as triacylglycerol biosynthesis (e.g., Mersmann, 1986). Thus, although the physiology and pharmacology of an enzymatic step or of a pathway may be accurately described by the results, the quantitative use of the rates is not appropriate. Second, the observed results may stem from a particular addition or deletion to the incubation medium; for example, insulin usually will potently inhibit lipolytic rates (Mersmann and Hu, 1987) or the endogenous generation of adenosine will stimulate the adipocyte adenosine A_1 receptor to inhibit adenylyl cyclase (Carey, 1995). Third, the concentration of hormones or drugs (agonists and antagonists) may be pharmacological, and thus the effects may not represent the physiological response in vivo. The concentrations in experiments in vivo may also exceed those attained with the indicated use of the compound. A good example of this is the exceptionally high concentrations of compounds used in toxicology studies, wherein, in some cases, the concentration far exceeds the capacity of the detoxification systems. The results represent a totally artificial situation in which the compound is studied at concentrations that exceed the organism's ability to detoxify and eliminate the compound in the usual manner.

Experimental Design In Vivo. The minute details of the design of an experiment in vivo may greatly influence the outcome. Most of us carefully examine the more obvious aspects of design when dissecting the results of an experiment or designing a new experiment. However, many of the subtle components are often overlooked or not reported. Manuscripts written today do not expound the details of methodology as in the middle decades of this century; we pay a price for the expansion of science in that there must be a conservation of space in the literature. Thus, the literature contains diverse results that seem to come from the same experimental design; many factors such as animal age, feed composition, feeder design, feeding pattern, temperature, humidity, air flow in a building (influencing temperature, humidity, dust, toxic gasses, etc.), waste disposal system, season, breed/line/source, cage design, animal density in cages or pens, and randomization or placement of cages or pens in a room or building may influence the results. These multiple design variables mandate experiments in vivo or in vitro must be duplicated in another laboratory to be accepted by the scientific community, including the regulatory organizations. Even so, with so many intangibles, it may be difficult to duplicate experiments, particularly to quantitatively replicate results. It is well established in the commercial drug business that a large number of trials with a single drug will produce highly variable quantitative results, including some negative (no effect) results. Some investigators are a bit too quick to judge the work of others as poorly executed when the results differ from the expected or from their own.

Ligand Delivery. The pharmacodynamic and pharmacokinetic characteristics of a drug may vary with species, breed/line, diet, and climatic conditions (could modify metabolic pathways in the animal). Many factors influence the pharmacodynamics and pharmacokinetics of an agonist, including 1) drug formulation, route of administration, and dose; 2) drug stability in the feed, the gut, the adipose tissue or muscle if injected, and the blood stream; 3) drug absorption including transport in the venous or lymphatic system; 4) drug metabolism including activation, degradation, and protein/binding; 5) drug delivery to the active site(s) including blood flow and cell permeability of capillaries and target cells; 6) receptor subtypes, concentration and extent of inactivation; and 7) drug excretion. Given the extensive number of qualifying factors, it should be apparent that extrapolation of results for a given drug across species is not appropriate. Given the many potential modifiers of the drug effect, it is not surprising that the quantitative effects observed in different drug trials, even when using the same design, in the same species, are widely divergent. Qualitative support of the results of others is more common than quantitative replication.

Species Variation in Receptor Structure. Results from comparative biology clearly indicate the primary amino acid sequence of a protein differs across species. If the amino acid substitutions are in areas of the protein not critical for function, there will be little modification of function. Also, amino acid substitutions may be by a similar amino acid (e.g., two acidic amino acids, such as aspartic acid for glutamic acid) so that function is not greatly altered. However, in other cases, the amino acid substitution may markedly modify function because the location is critical to function, and there are major differences between the

original and substituted amino acid (e.g., an acidic for a basic amino acid, such as glutamic acid for lysine). There may also be extensive modification of the primary structure by regions of deletion, repetition, or insertion; if the modifications are exceptionally great the function of the protein may be lost.

Although not many species have been analyzed for β-AR amino acid sequence (or more likely the nucleotide sequence extrapolated to the amino acid sequence), there are enough data available for the sequences of each of the three β-AR subtypes to establish sequence variability. First, in a given species, the β_1-AR, β_2-AR, and β_3-AR have approximately 40 to 50% homology with each other; the greatest conservation of sequence is in the transmembrane domains, several of which contribute to form the active site of these receptors. A given β-AR subtype has approximately 75% (or greater) homology across species. For example, the bovine β_3-AR has approximately 85% amino acid sequence homology with human β_3-AR and 75% homology with rat and mouse β_3-AR (Pietri-Rouxel et al., 1995).

Despite the retained similarity in structure, the pharmacological properties of the β_3-AR from these species are quite different (Table 4). Cloned bovine, human, and mouse β_3-AR individually transfected into Chinese hamster ovary cells have strikingly different pharmacological properties. Propranolol is an antagonist for mouse receptors, but a partial agonist for human and bovine receptors, whereas bupranolol is an antagonist for mouse and human receptors but a partial agonist for bovine receptors. Many other pharmacological differences between these closely related receptors are documented (Pietri-Rouxel et al., 1995). Given this concrete demonstration of modification of receptor-ligand interaction (even to the extent that a given compound is an agonist for a receptor subtype in one species but an antagonist for the same receptor subtype in another species), it is not surprising different effects are observed in different species with the oral administration of the same β-AR agonist.

Although there is no experimental observation as yet, there could be isoforms of a single β-AR subtype in different tissues in a single species; for example, the primary amino acid sequence (two different genes) for the predominant β-AR in two different tissues in the same species could differ, leading to diverse pharmacological properties in the two tissues, even though both receptors would be classified as the same β-AR subtype.

Cellular Distribution of β*-Adrenergic Receptor Subtypes.* The exact proportion of each β-AR subtype present on the surface of a specific cell type may vary across species. This would modify the response of that tissue to stimulation by an agonist even if the receptors had an identical sequence with the same receptors in another species. For example, rat adipocytes have predominantly β_3-AR, but this is not true for human adipocytes (Langin et al., 1995). As already mentioned, individual β-AR subtypes may be transcriptionally regulated or regulated through desensitization to produce variation in the cell surface population of subtypes. It has recently been demonstrated that individual adipocytes from a single depot have different distributions of the three β-AR subtypes; if this observation is supported, it adds another layer of complexity to the regulation of β-AR subtypes (Seydoux et al., 1996).

Table 4. Species variation in pharmacology of β_3-adrenergic receptors (AR)[a]

Ligand and species of β_3-AR	Effect[b]
Propranolol	
Mouse	Antagonist
Human	Partial agonist
Bovine	Partial agonist
Bupranolol	
Mouse	Antagonist
Human	Antagonist
Bovine	Partial agonist

[a]Adapted from Pietri-Rouxel et al. (1995).
[b]Adenylyl cyclase activity measured in cloned β_3-AR transfected into Chinese hamster ovary cells.

Further complexities involve modulation of the coupling of the receptors to the intracellular signaling system. Ligand-binding to the receptor is only the initiating event; detailed studies of the modification of receptor coupling as a mechanism for control of cellular function are few.

Implications

Some compounds that stimulate β-adrenergic receptors (β-AR), i.e., β-adrenergic agonists increase muscle mass and decrease fat mass when fed to growing cattle, pigs, poultry, and sheep. Because the β-AR are present on almost every mammalian cell type, the mechanism by which modification of growth occurs is probably complex. It may include modulation of blood flow and secondary effects of other hormones, the release of which is controlled by β-AR. Regardless of complexity, the mechanism for a given β-AR agonist, in a given species, probably involves the direct mechanism of binding to β-AR present on the surface of skeletal muscle and adipocytes. There are species differences in the structure and pharmacology of the β-AR, in the β-AR subtypes present in individual tissues, and in the metabolism and distribution of individual β-AR agonists.

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